REMARKS

Claims 1 – 3, 6 – 9, 11 – 12 and 18 are pending in the application. Claims 5, 6, 10 and 13-17 have been previously cancelled. Claims 1, 6, 12 and 18 have been amended. No new claims have been added. No new matter has been added.

Any cancellation of the claims should in no way be construed as acquiescence to any of the Examiner's rejections and was done solely to expedite the prosecution of the application. Applicant reserves the right to pursue the claims as originally filed in this or a separate application(s).

The Examiner has withdrawn the rejection under 35 USC §112, first paragraph.

The Examiner has withdrawn the rejection to claims 1 – 9, 11, 12 and 18 under 35 USC §103(a) in favor of a new rejection.

Claim Rejections 35 USC §103(a)

The Examiner has rejected claims 1 – 3, 6 – 9, 11 – 12 and 18 as being unpatentable over the combination of Marcato et al. (Infection and Immunity vol. 70 p.1279 (3.2002) in view of LaCasse et al.(Blood vol. 88 p.1561 (1995)) and Strockbine et al. (J Bacteriology vol. 170 p.1116), Accession Number 2002:397002, Green (US 2002/0081307) and Applicant's admission on page 6, lines 1 – 2 of the specification. Applicants respectfully disagree.

The present claims recite a method of reducing, or inhibiting invasiveness and metastasis of tumor cells in a subject, wherein the tumor cells produce Gb3, comprising administering to the subject a therapeutically effective amount of a **StxB1 B-subunit of Shiga toxin.**

Accordingly, Applicants have particularly chosen **Stx1B** for use in the methods as claimed. Applicants teach that there are a number of Shiga toxin variants and subunits, for example at page 6, beginning at line 30 of the present disclosure:

> The sequences of numerous Shiga toxin variants and subunits are known in the art. For example, the Shiga toxin 1

B-subunit from the E. coli O157:H7 strain is set forth in GenBank Accession Nos. 32400300 and 32400303, the Shiga toxin 2 B-subunit from the E. coli O157:H7 strain is set forth in GenBank Accession No. 13359150, the Shiga toxin 1 A-subunit is set from the E. coli O157:H7 strain is set forth in GenBank Accession Nos. 32400299 and 32400302, and the Shiga toxin 2 A-subunit from the E. coli O157:H7 strain is set forth in GenBank Accession No.15718405.

Among all of these variants and subunits, Applicants have particularly chosen **Stx1B**.

Further, Applicants demonstrate that StxB1 can be used for reducing, or inhibiting invasiveness and metastasis of tumor cells in a subject, wherein the tumor cells produce Gb3. For example, in Example 7 beginning at page 43, Applicants show that Stx1B alone to cause apoptosis in human colon cancer cells. In these experiments, Applicants show that "Stx1B internalization caused massive DNA fragmentation in Caco-2 cells, compared to intact non-fragmented DNA form cells not exposed to Stx1B (lane b) or incubated with CTB (Cholera Toxin B subunit) (lane e)."

In Example 8, on pages 43 - 44, Applicants show that Stx1B selectively causes apoptotic death in cells expressing Gb3.

In Example 10 beginning at page 45, Applicants use a nude mouse xenograft model to determine if StxB1 has apoptotic effects on growing tumors. Applicants show in Figure 8 that Stx1B injections significantly inhibited tumor growth in nude mice

The Marcato reference does not teach or suggest all the limitations of the instant claims. In particular, the Marcato reference does not teach or suggest a method of reducing, or inhibiting invasiveness and metastasis of tumor cells in a subject, wherein the tumor cells produce Gb3, comprising administering to the subject a therapeutically effective amount of a Stx1B B-subunit of Shiga toxin.

Marcato et al. teach the use of the cloned shiga toxin B (Stx2 B) subunit to induce apoptosis in Burkitt Lymphoma B-cells. Nowhere does Marcato teach inhibiting invasiveness and metastasis of tumor cells in a subject using Stx1B. In fact, Marcato et al. teaches away from using Stx1B in the methods as claimed. At page 1279 Marcato teaches that "the present study describes the results of our investigations into this

activity which, in our laboratory, appears to be more potent in the Stx2B than in the Stx1B subunit."

Nowhere in the Marcato reference is there teaching or suggestion of a method of reducing, or inhibiting invasiveness and metastasis of tumor cells in a subject, wherein the tumor cells produce Gb3, comprising administering to the subject a therapeutically effective amount of a Stx1B B-subunit of Shiga toxin as claimed.

None of the LaCasse, Strockbine or Greene references cure the defects of the Marcato reference. None of the references, alone or in combination, teach or suggest a method of reducing, or inhibiting invasiveness and metastasis of tumor cells in a subject, wherein the tumor cells produce Gb3, comprising administering to the subject a therapeutically effective amount of a StxB1 B-subunit of Shiga toxin.

As previously discussed, the LaCasse reference is directed to the use of shiga like toxin (SLT-1) in human bone marrow (BM) purging. LaCasse uses Shiga Like Toxin (SLT-1) which kills cells by inhibiting protein synthesis. (p.1561). The purpose of the study described by LaCasse "was to establish the potential of a natural toxin (SLT-1) in purging B-cell lymphomas from BM." (p.1563).

LaCasse does not teach or suggest a method of reducing, or inhibiting invasiveness and metastasis of tumor cells in a subject using Stx1B.

The Examiner argues that "LaCasse et al disclose treatment of human B cell lymphoma from bone marrow in mice using Shiga-like toxin 1 (and) also discloses that the toxin was administered after the cancer is present." (Office Action, p.4). The Examiner argues further that "(o)n page 6 of the specification, Applicant admits the toxins are known to bind to Gb3 expressing cells, therefore it is expected that the cells of the reference are Gb3 expressing cells." (Office Action, p.4).

It would not have been obvious to one of ordinary skill in the art that StxB subunit can also be used to inhibit apoptosis in vivo, as argued by the Examiner on page 5 of the Office Action. The teachings of Marcato would not motivate one to use Stx1B in place of SLT-1, as taught by LaCasse. The teachings of the cited art, when combined, do not result in the claimed invention.

Accordingly, Applicants request that the rejection be withdrawn and the claims allowed.

CONCLUSION

In view of the above amendment, applicant believes the pending application is in condition for allowance.

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Respectfully submitted,

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